

LETTERS AND
CORRESPONDENCE

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Pure Red Cell Aplasia (PRCA) With Thymoma: A Possible Distinct Clinical Entity Distinct From Large Granular Lymphocyte (LGL) Leukemia

To the Editor: Semenzato et al. proposed new criteria for large granular lymphocyte (LGL) leukemia because patients with low levels of GL were similar to those with levels greater than 2,000 GL/ μ l [1]. LGL leukemia is a GL proliferative disease often accompanied by pure red cell aplasia (PRCA). T-cell LGL leukemia is a clonal disease, and the phenotype of leukemic cells is CD3⁺4⁺8⁺.

PRCA with thymoma is often associated with peripheral T cell expansion. We previously reported a case of PRCA with thymoma in which the T-cell receptor β of both the thymus and peripheral mononuclear cells was rearranged [2]. Thymoma and PRCA may be caused by T-cell clonal expansion, leukemia. We examined four patients with PRCA and thymoma (Table I), one of which was previously reported. Three of the four showed CD3⁺4⁺8⁺ lymphocytosis and two exhibited T-cell receptor β rearrangement of peripheral mononuclear cells. Patient 2 showed low levels of lymphocytes and the mononuclear cell's CD4/8 ratio of patient 3 was greater than 1. Due to the low levels of abnormal cells and low sensitivity of Southern blot analysis, we could not confirm the clonality of these two cases. According to treatment of PRCA with thymoma, the response rate of thymectomy was 12% [3]. This limited effectiveness of thymectomy indicated that thymoma did not play a causative role in PRCA.

Loughran reported 129 patients with LGL leukemia [4] and Oshimi et al. examined 33 patients [5]. Neither study noted a complication of thymoma. PRCA with thymoma had been considered a distinct clinical entity from LGL leukemia. The old diagnostic criteria for LGL leukemia was "greater than 2,000 GL/ μ l." The normal value of GL is $223 \pm 99 \mu$ l [4]. Three of the 4 patients with PRCA and thymoma demonstrated levels greater than the mean + 3 SD (Table I). However, it is unknown whether or not a distinction between 'PRCA with thymoma' and 'PRCA with LGL leukemia' exists. Patients with LGL leukemia showed an indolent clinical course, and immunosuppressive therapy with cyclosporin A or low-dose cyclophosphamide was effective for the clinical symptoms. In contrast, patients with PRCA with thymoma were treated with more aggressive therapy such as surgical resection, intensive chemotherapy, or radiation. As the effectiveness of surgical resection for PRCA with thymoma is limited, PRCA with thymoma should be initially treated with immunosuppressive therapy.

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Factor V (His 1299 Arg) in Turkish Patients With Venous Thromboembolism

To the Editor: Recently, a genetic component in the factor V (FV) gene that contributes to activated protein C (APC) resistance both in the presence

TABLE I. Laboratory Findings of Four Patients With PRCA and Thymoma

| Patient's No. | Age | Sex | No. of (/ μ l) | | | Surface marker of PBMC (%) | | | | | Clonality of PBMC | |
|---------------|-----|-----|--------------------|-------------|-----|----------------------------|-----|-----|-----|-------------|-------------------|---------------|
| | | | WBC | Lymphocytes | GL | CD2 | CD3 | CD4 | CD8 | CD4/8 ratio | | |
| 1 | 55 | M | 9,110 | 3,030 | 853 | 94 | 88 | 20 | 68 | 0.29 | TCR β | Rearrangement |
| 2 | 56 | M | 4,000 | 1,680 | 218 | 92 | 86 | 39 | 47 | 0.83 | TCR β | Germ line |
| 3 | 66 | F | 35,900 | 3,700 | 710 | 81 | 72 | 43 | 33 | 1.3 | TCR β | Germ line |
| 4 | 44 | M | 14,100 | 9,020 | 720 | 88 | 90 | 38 | 55 | 0.69 | TCR β | Rearrangement |

TABLE I. Frequency of the Factor V Gene 4070 G-A Genotype in the Turkish Population

| | <i>n</i> | Heterozygous 1691 G-A | 1691A frequency | FV4070A | OR (CI 95%) | Chromosome | FV4070 G allele | % | G allele frequency | OR (CI 95%) |
|----------------------------------|----------|--------------------------|--------------------|---------|--------------|------------|--------------------|------|-----------------------|----------------|
| VTE | 148 | 39 (3) ^a | 0.1418 | 26.3 | 1 | 296 | 15 | 10.1 | 0.05 | 1 |
| Controls (FV 1691 A excluded) | 82 | 7 | 0.0426 | 8.5 | 3.1(0.2–7.4) | 164 | 7 | 8.5 | 0.04 | 0.8(0.16–0.94) |
| VTE | 109 | — | — | — | | 218 | 13 | 11.9 | 0.06 | 1 |
| Control | 75 | — | — | — | | 150 | 7 | 9.3 | 0.04 | 0.8(0.7–1.2) |

^aHomozygous.

and in the absence of FV Leiden was reported [1,2]. This highly conserved FV gene haplotype was marked as R2 polymorphism, an A to G alteration at position 4070 in exon 13 that predicts the His 1299 Arg substitutions [3]. R2 haplotype was reported in populations from Somali, Southern Indians, Italians, and Greek Cypriots with a frequency of 0.075 [2,3].

Our aim was to determine the frequency of this mutation in Turkish patients with venous thromboembolic disease (VTE). One hundred forty-eight patients with VTE and 82 healthy unrelated individuals from Ankara without any familial history of thrombosis and stroke were included in the study [4].

DNA was extracted by conventional methods and polymerase chain reaction of exon 13 of the Factor V gene was performed according to the previously described method using primers 5'-CAAGTCCTTCCCCACAGATATA-3' (nt 3,579–3,600) and 5'-AGATCTGCAAAGAGGGGCAT-3' (nt 4,280–4,261). Amplification was performed for 35 cycles with annealing temperature of 57°C (Ericomp, USA). Amplified DNA was digested with *RsaI* enzyme (Promega, Madison, WI) at 37°C and subjected to 2% agarose gel electrophoresis [3]. FV 1691 G-A mutation was performed according to previously described method [4]. Individuals with prothrombin 20210 G-A mutation were excluded [5].

The results of the distribution of FV 4070 mutation are shown in Table I.

Fifteen (10.1%) of the 148 Turkish VTE patients carried 4070 G allele in the heterozygous condition (Frequency of the G allele was 0.05). Of the 82 healthy controls, 7 (8.5%) were found to carry this mutation (Table I). The difference between the two groups was not significant ($P = 0.9$).

After excluding subjects with FV R506Q mutation, His 1299 Arg was also not a risk factor for VTE in the remaining patients, with an odds ratio of 0.8 (95% CI: 0.16–0.94; $P = 0.9$), demonstrating that this mutation is not a risk factor by itself.

As a conclusion, we can say that FV 1299 His-Arg mutation is prevalent in our population and does not have any effect on the occurrence of VTE.

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Hydroxyurea-Induced Gangrene of the Toes in a Patient With Chronic Myelogenous Leukemia

To the Editor: Gangrene of the toes and fingers has been reported in patients with myeloproliferative disorders including polycythemia vera, essential thrombocythemia, chronic myelogenous leukemia (CML), myelofibrosis, and myeloid metaplasia [1]. An increase in circulating platelets is associated with thrombotic phenomena affecting the arterial and venous circulation. Herein we report on a patient with CML who showed normal platelet count values and developed gangrene of the toes during hydroxyurea and interferon (INF) α treatment (Fig. 1).

A 53-year-old woman with Ph1-positive CML in chronic phase received 1.5 g of hydroxyurea daily and IFN α at 1×10^7 units 3 times per week. Since she developed IFN-related retinopathy one month later, IFN α dosage



Fig. 1. Gangrenous changes in toes of patient receiving hydroxyurea treatment. Amputation was necessary.

was decreased to 3×10^6 units once a week. After 3 years of continuous therapy, she developed ischemic changes and gangrene of the first through fifth toes of the right foot and the first and second toes of the left foot. Results of a blood workup were as follows: hemoglobin level, 9.9 g/dL; leukocyte count 1800/ μ L (neutrophils 21.8%, lymphocytes 28.7%, monocyte 38.6%, eosinophils 1.0%, basophils 6.9%, metamyelocyte 1.0%, myelocyte 2.0%); and platelet count, 22.6×10^4 / μ L. The patient's coagulation factor levels, serum cholesterol level, and serum triglyceride level were within normal limits. Arteriography revealed neither occlusion nor arteriosclerotic change of the major arteries of both legs, including anterior tibial arteries, posterior tibial arteries and peroneal arteries. After transmetatarsal amputation of 2 right toes and all left toes, she developed patchy cutaneous gangrene and extremely painful ulcers on both amputated edges, both heels, and left lateral malleolus. The ulcer increased in size to 1 cm \times 1.5 cm. Histological examination of amputated tissues and cutaneous ulcers showed nondiagnostic changes as follows: subepidermal edema and focal hyalinization of blood vessels but no perivascular lymphocytic inflammation. Hydroxyurea treatment was discontinued when its association with the gangrene was identified. The patchy cutaneous gangrene and ulcer healed over the course of 3 months. IFN α has been continued at the same dosage since IFN α withdrawal for a few weeks disturbed the control of leukocyte count but brought about no change of the gangrene.

Thrombotic complications, including gangrene of the toes and fingers, have been reported in the patients with myeloproliferative disorders when associated with thrombocytosis [1]. However, our patient had a platelet count of 69.1×10^4 / μ L only at the first presentation, but it decreased to the normal value after hydroxyurea and IFN α treatment started. IFN α has been also documented to cause the ischemic manifestations, including Raynaud's phenomenon [2], but the frequency of gangrene is very low [3]. Recently, an intimate association between hydroxyurea treatment and cutaneous ulceration of the legs has been reported by several authors [4,5]. The most common ulcer site was the malleoli, and ulcers usually require cessation of hydroxyurea therapy. Although they describe no patient with gangrene requiring amputation, leg ulceration in our patient after amputation has the same clinical features similar to that reported by them. Gangrene and ulcer healing after cessation of hydroxyurea treatment suggest an association between hydroxyurea therapy and the development of the gangrene in our patient.

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cestry. The disease is inherited as a Mendelian recessive trait and leads to excessive absorption and tissue deposition of iron, resulting in organ damage. The molecular basis of this disease was established in 1996 when a mutation in a novel HLA-like gene, now termed HFE, was found in the vast majority of cases of HH [1]. This finding provided the possibility of a genetic diagnosis of HH rather than relying on surrogate markers such as serum ferritin or transferrin saturation, which may not become abnormal in affected individuals until later in life. The usual approach to genetic testing relies on polymerase chain reaction (PCR) amplification of the region of exon 4 of the HFE gene containing the HH mutation, followed by mutation detection by restriction enzyme digestion (the mutation creates an *RsaI* restriction site) or techniques such as single-stranded conformational polymorphism (SSCP).

During routine testing for the HH-associated C282Y mutation using a previously described method [2] we observed for some samples that different SSCP patterns and *RsaI* digest patterns occurred depending on the type of Taq polymerase used for the PCR amplification. Specifically, two polymerases yielded a heterozygous SSCP or digest patterns with an abnormally faint band for the normal allele, while a third "hot start" modified Taq polymerase that possesses 5'-3' exonuclease activity yielded a homozygous mutant allele pattern. It occurred to us that one possibility for this finding was a polymorphism in the DNA sequences to which the PCR primers hybridize. To test this hypothesis we synthesised new primers (forward, 5'-TATTCCTTCCTCCAACC-3'; reverse, 5'-ATCCCTA-ACAAAGAGCA-3') that would amplify the region containing the earlier target region including primer binding sites and proceeded to sequence this. We determined that the previous aberrant results were due to a novel G to A polymorphism within intron 4 of the HFE gene (genomic sequence 5'-GGTTg/aAGAGGAGTGCCTGAG-3') at the binding site of the original reverse PCR primer. We have named this polymorphism CEM after the initials of the original patient. Analysis of 22 patient samples sent for HH genetic testing, which lacked other recognized HFE mutations (C282Y, H63D, S65C), for the CEM polymorphism showed a heterozygous frequency of 40%. Among 20 randomly selected blood samples, where HH was not suspected, 18% were heterozygous for CEM. No homozygotes for CEM were found in either group. Of 194 patients heterozygous for the C282Y mutation, 12.4% carried the CEM polymorphism. Interestingly, to date the C282Y mutation and the CEM polymorphism have not been observed on the same allele.

Many studies have examined the frequency of C282Y mutation in a range of populations. In some studies, the ratio of homozygotes to heterozygotes has been higher than that predicted by the Hardy-Weinberg equilibrium. We can now explain this anomaly on the basis of the CEM polymorphism which results in an overestimation of homozygotes. In view of these findings, we recommend that the genetic diagnosis of HH be performed with thought to the effects of CEM on PCR amplification and that consideration be given to method modification and reviewing patient samples previously found to yield a homozygous C282Y result.

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Incorrect Diagnosis of Hereditary Hemochromatosis

To the Editor: Hereditary hemochromatosis (HH) is a common inherited disease of iron metabolism among individuals of northern European an-

- MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399–408.
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Hypercalcemia and Adrenal Insufficiency in a Patient With Myelofibrosis

To the Editor: We describe a 44-year-old female with myelofibrosis who presented with clinical and laboratory features of hypercalcemia, sclerotic and lytic bone lesions, together with adrenal insufficiency. She first presented 5 years ago with a mild leucocytosis of $21 \times 10^9/l$ and left shift on a routine blood count. She was a known diabetic who had been on daily insulin for more than 10 years with reasonably good control. Physical examination revealed 8 cm of splenomegaly. Bone marrow biopsy was mildly hypercellular with myeloid hyperplasia and pronounced fibrosis on reticulin staining, consistent with myelofibrosis. Karyotype was normal.

Over a 4-year period, her spleen enlarged progressively and she became anemic, requiring regular transfusions. Subsequently, she presented with drowsiness, polyuria, and polydipsia, with a corrected serum calcium of 4.0 mmol/l. She responded to intravenous fluids, lasix, and pamidronate both clinically and biochemically. Skeletal X-ray survey showed widespread lytic and sclerotic lesions affecting the long bones, lumbo-sacral spine, and pelvis (Fig. 1). Serum PTH was normal as were serum protein EPG, and chest X-ray. She required a total of three infusions of pamidronate over a 7-week period and maintained a normal serum calcium subsequently.

Over the next 2 months her insulin requirements fell steadily and she experienced occasional hypoglycaemic episodes. She reported increasing fatigue and lassitude that responded only partially to blood transfusion. Furthermore, she was noted to become progressively hyponatremic (127 mmol/l), hyperkalemic (6.0 mmol/l), with a creatinine of 114 $\mu\text{mol/l}$. Blood sugar levels fell to between 1.6 and 6.0 mmol/l in the absence of any exogenous insulin, diuretics, or prednisone. Her systolic blood pressure was 120 mmHg lying and 100 mmHg standing. A short synacthen test demonstrated cortisol values of 366 mmol/l at baseline and 507 mmol/l at 60 min, consistent with adrenal insufficiency. Abdominal CT scan confirmed a spleen of 20 cm in length and massive hepatomegaly, but without obvious adrenal gland enlargement. She was treated with hydrocortisone (tapering regimen) and fludrocortisone (0.05 mg daily), which fully corrected her biochemical indices as well as dramatically improving her clinical

symptoms. However, over the ensuing weeks her transfusion requirements increased steadily, and she became progressively more thrombocytopenic with platelet counts less than $10 \times 10^9/l$. Following a presentation with massive melena, requiring significant blood transfusion, she became obtunded and died soon after.

Hypercalcemia in association with sclerotic and lytic bone lesions has been reported only occasionally in myelofibrosis, primarily in patients reporting prolonged periods of immobilisation [1–4]. One proposed biochemical mechanism is that of ectopic production of 1,25-dihydroxyvitamin D₃, related to ongoing clonal marrow expansion [1]. In the present case her hypercalcemia was not associated with causes such as sarcoidosis, hyperparathyroidism, other malignancy, or even prolonged bedrest, although vitamin D₃ levels were not measured.

Adrenal involvement in myelofibrosis has been described elsewhere and is thought to be secondary to extramedullary hemopoiesis in the adrenal glands [5]. However, we could find no cases of adrenal insufficiency reported in the literature in myelofibrosis. The cause of adrenal insufficiency in this case is not clear; however, despite the normal CT scan appearance, it is likely to be due to extramedullary hemopoiesis although other causes such as bleeding cannot be fully excluded.

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Fig. 1. X-ray of long bones demonstrates significant sclerotic and lytic areas.

Paraneoplastic Pemphigus in Two Patients With B-Cell Non-Hodgkin's Lymphoma: Significant Responses to Cyclophosphamide and Prednisolone

To the Editor: Paraneoplastic pemphigus (PNP) is an autoimmune disease characterised by painful mucosal ulcerations and polymorphous skin lesions in association with an underlying neoplasm, most commonly lymphoma [1,2]. We describe two patients with B-cell non-Hodgkin's lymphoma (NHL) who developed PNP which responded dramatically to a combination of cyclophosphamide and prednisolone.

The first patient was a 73-year-old female who presented with widespread peripheral and abdominal lymphadenopathy. Biopsy confirmed a follicular small cell NHL. Over the ensuing 3 years she was treated with a number of regimens including CVP (cyclophosphamide, vincristine, and prednisolone), chlorambucil and prednisolone, and finally CNP (cyclophosphamide, novantrone, prednisolone). Each of these regimens induced a good partial response, however, she soon developed a painful conjunctivitis with epiphora, a sore throat, painful buccal ulcers, haemorrhagic crusted lips, and a widespread papulosquamous erythematous eruption over her face, trunk, and limbs. Skin biopsy showed hyperkeratosis, liquefaction at the dermal/epidermal interface with lymphocyte satellitosis around de-

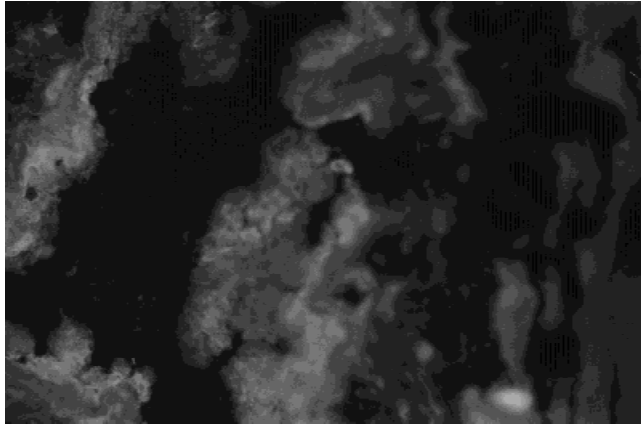


Fig. 1. Positive indirect immunofluorescence on rat bladder represents the diagnostic test for PNP.

generate keratinocytes. Indirect immunofluorescence of patient serum to rat bladder epithelium was positive consistent with PNP (Fig. 1). She was commenced on oral prednisolone 100 mg and cyclophosphamide 150 mg daily. Within 14 days, her conjunctivitis, oropharyngeal ulceration, and truncal rash had dramatically improved and fully resolved by 4 weeks. She was maintained on cyclophosphamide 100 mg daily together with a tapering regimen of prednisolone; however, she subsequently developed CMV oesophagitis, necessitating rapid reduction in her immunosuppression. Although the PNP did not return, her lymphoma progressed rapidly over the next 2 months, leading to her ultimate demise.

The second patient was a 64-year-old female who was diagnosed with diffuse large B-cell NHL on biopsy of a large paraaortic mass. She was treated with six courses of CHOP chemotherapy and involved-field abdominal radiotherapy. However, within 8 months her disease had relapsed and she was treated with oral dexamethasone 40 mg and etoposide 100 mg daily for 3 days every 21 days, achieving a partial response after four courses. Within 4 months, however, she presented with severe buccal ulceration, haemorrhagic crusting of lips and nasal mucosa, painful conjunctivitis with epiphora, and a widespread papulosquamous eruption with blistering. Skin biopsy showed an atrophic epidermis with prominent apoptosis and basal acantholysis together with transepithelial migration of mixed inflammatory cells and spongiosis. Indirect immunofluorescence to intercellular cement was positive at moderate titre consistent with pemphigus. She was commenced on oral prednisolone 100 mg plus cyclophosphamide 150 mg (subsequently 100 mg) orally daily. Within 10 days her symptoms had begun to improve, and by 21 days her oropharyngeal ulceration, conjunctivitis, and rash had virtually resolved. Her prednisolone dosage was tapered over the ensuing weeks while she remained on oral cyclophosphamide. Despite this, however, within 3 months her lymphoma progressed rapidly and she subsequently died as a result.

Treatment of PNP is difficult. Cytotoxic immunosuppressives and prednisolone are the mainstay of current systemic therapy [2]. Other agents such as azathioprine [3], cyclosporin [4], mycophenolate, and even plasmapheresis have been used with variable results [5]. In these two patients with lymphoma, a combination of oral cyclophosphamide and prednisolone lead to a dramatic reduction in the symptoms and severity of their PNP.

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Abdominal Venous Thrombosis With Prothrombin Gene Mutation

To the Editor: More than 50% of patients who develop spontaneous deep venous thrombosis (DVT) can be demonstrated to have one or more recognized thrombophilic disorder(s). The majority of these defects are genetic in nature associated with mutation in the genes encoding either factor V or factor II (prothrombin). Factor V Leiden is the most commonly observed mutation. Its frequency in European populations is 3–8% [1]. It is found in approximately 15–20% of patients diagnosed with a first episode of DVT, and its incidence increases up to 50% in patients with recurrent venous thrombosis [1].

The G20210A mutation is a defect found in the 3'-untranslated region of the prothrombin gene. The single amino acid replacement (glycine to alanine) enhances the secretion of prothrombin which leads to a hypercoagulable state with increased risk of peripheral venous thrombosis [1]. This mutation is found in 2% of the general population and rises to 6% in patients with a single DVT. More significantly, it is present in 18% of patients with either a personal or family history of thrombosis [1]. Thrombotic events due to the prothrombin gene mutation predominantly manifest in the deep venous circuits of lower extremities [2].

Our patient is a 51-year-old Caucasian male presented to his primary physician's office in October 1998 with a gradual onset of progressive midepigastria pain aggravated by food intake. He had no additional symptomatology and was treated with omeprazole. One week later, more severe abdominal pain developed and an abdominal ultrasound showed extensive abdominal vein partial thrombosis including portal, superior mesenteric, and splenic veins. Both CT scanning and MRI of the abdomen (Fig. 1) confirmed the thrombosis.

Heparin was started followed by coumadin. The abdominal discomfort resolved in 2 days, and he was referred to our institution for further evaluation. Sucrose test, lupus anticoagulant, protein C and S, antithrombin III, and factor V Leiden were obtained and all were normal with the exception of a mild decrease in protein S, consistent with the patient's coumadin therapy. PCR analysis was positive for the heterozygous form of the prothrombin gene mutation G20210A. Screening of the patient's close relatives did not reveal any other individuals with the same mutation. The

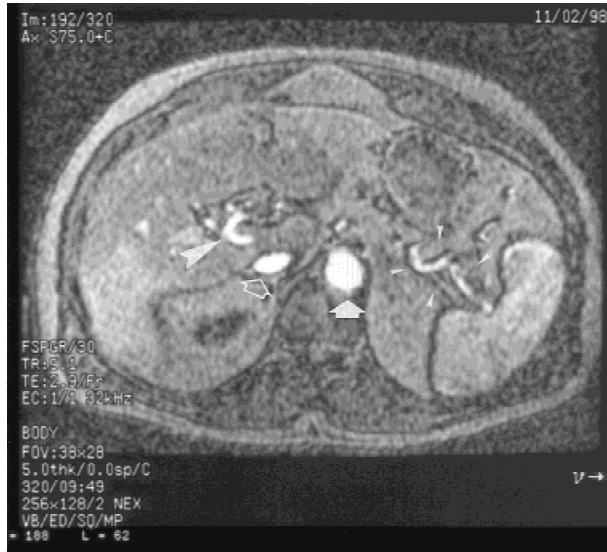


Fig. 1. Axial gradient echo image (9.1/2.9/30) through the abdomen shows partially obstructing thrombus in the portal vein. The aorta, inferior vena cava, portal vein with partially obstructing thrombus, and splenic artery and vein are indicated by the large solid arrow, large lined arrow, large arrowhead, and small arrowheads, respectively.

patient has continued on coumadin with INR target of 2.5–3.5 without any complications or evidence of new thrombus.

To our knowledge, only four cases of the G20210A gene mutation that presented with intra-abdominal vein thrombosis have been reported [3–5]. Two of these patients had additional risk factors, chronic estrogen replacement treatment [5] in one and polycythaemia [3] vera in the other, that may

have contributed to thrombus formation. The other two cases involved hepatic vein [4] or superior mesenteric vein [5] thrombosis. Our patient is the first G20210A heterozygote we are aware of who has been documented to have extensive intra-abdominal partial thrombosis including portal, superior mesenteric, and splenic veins (particularly around the confluence) in the absence of other factors contributing to hypercoagulability. We suggest that prothrombin G20210A analysis should be included in the differential diagnosis of intra-abdominal venous thrombosis in all patients, regardless of whether there is a personal or family history of venous thrombotic disease.

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